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MECHANICAL PROPERTIES OF PERIVITELLINE FIBERS OF SEA URCHIN EGGS AS STUDIED BY APPLICATION OF CENTRIFUGAL AND ELECTROPHORETIC FORCES

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Abstract

Fertilized sea urchin eggs are concentrically located in the perivitelline space. However, after centrifugation or treatment with Ca-free sea water, most eggs came to rest on the bottom of the perivitelline space, and few fibers were detected within the perivitelline space by differential interference microscopy. In normal fertilized eggs with the fibers, the egg shifted to the anode under an electric field and returned to its original position after the field was shut off. In centrifuged eggs without the fibers the recovery to the concentric position was not achieved. Such invariable coincidence of the perivitelline fibers and the concentric position of the eggs shows that the fibers are the structure supporting the egg in the central portion of the perivitelline space.

The mechanical properties of these fibers were studied by application of centrifugal and electrophoretic forces. The results indicate that: 1. the fibers show an internal viscous resistance against stretching and 2. tension of the fibers is about 0.9×10^{-5} dyne which is large enough to support the fertilized egg in the perivitelline space.

Introduction

In a horizontal view of fertilized sea urchin eggs, the egg is situated at the center of the perivitelline space. On finding that the density of the egg is higher than that of the perivitelline fluid, Hiramoto (1954) predicted the presence in the perivitelline space of some unknown structure which supports the egg. He also noticed that by centrifugation or treatment with Ca-free sea water the egg settled to the bottom of the perivitelline space, and suggested lability of the structure.

In 1973 Sato *et al.* found, by differential interference microscopy, many thin fibers attaching the egg surface and emanating in all directions towards the fertilization membrane. They also stated that the fibers exist until the morula stage and that they are easily dissolved by treatment with trypsin, pronase, or urea. Thus it appeared that these fibers are the physical entity supporting the egg in position as predicted by Hiramoto.

In this paper, the mechanical properties of the fibers were studied by application of two kinds of external forces, centrifugal and electrophoretic forces. It will be shown that, though seemingly very fine and labile, the fibers are stiff enough to support the weight of the egg in the perivitelline space.

Materials and Methods

Fertilized eggs of the sea urchins, *Anthocidaris crassispina* and *Hemicentrotus pulcherrimus,* were used.

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For electrophoresis, the fertilized eggs were fixed in a gap (about 110 μm) between slide glass and cover glass. Both ends were closed by blocks of agar and connected to electrodes through agar bridges. Magnitude of electric current was changed by a direct current power supply (Mitamura Riken). In order to reduce electric conductivity of the medium so as to attain a definite electric field by weaker current, the fertilized eggs were suspended in sucrose-sea water solution (0.75 *M* isotonic sucrose solution/sea water ratio, 5:3; pH 7.9). The migration speed of the egg within the perivitelline space was determined by timing its transit over one (*A. crassispina*) or two (*H. pulcherrimus*) divisions of an ocular micrometer (one division corresponds to 5 μm) with a stopwatch. An 8 mm cinemicrocamera was also used to analyze the movement of the egg under the electric field.

Ca-free artificial sea water used in the present study was composed of 0.462 *M* NaCl, 0.009 *M* KCl, 0.048 *M* $MgCl_2$ and 0.006 *M* $NaHCO_3$.

The perivitelline fibers were observed by Nomarski differential interference microscope with a 40× objective (Olympus).

Centrifugal breakdown of the perivitelline fibers was routinely done 10–15 min after fertilization at 25°C.

Results

Centrifugation of fertilized eggs

When the fertilized eggs of *H. pulcherrimus* were centrifuged at 125 times gravity for 3–7 min, most of the eggs rested upon the bottom of the perivitelline space (Fig. 1). This observation suggests that a structure supporting the egg is broken by low-speed centrifugation. Centrifugation at 125 g for one min, which Hiramoto (1954) used to dislocate the egg, was not effective in the present preparation.

Observation of fibers within perivitelline space

The fibers within the perivitelline space were observed by differential interference microscopy (Fig. 2). As pointed out by Sato *et al.* (1973), they looked thinner than sperm tails (about 0.2 μm in diameter) of the sea urchin. The fibers link the egg to the fertilization membrane, and their arrangement is radiate and random. This organization of protoplasmic fibers persisted even after centrifugation for one min at 125 g. After centrifugation for more than 3 min, most eggs came to the bottom of the perivitelline space, and only a few fibers were detected in the perivitelline space. Therefore, it may be concluded that these fibers are the structure which keeps the egg in the central portion of the perivitelline space.

The fibers which had been broken by centrifugation were not formed again.

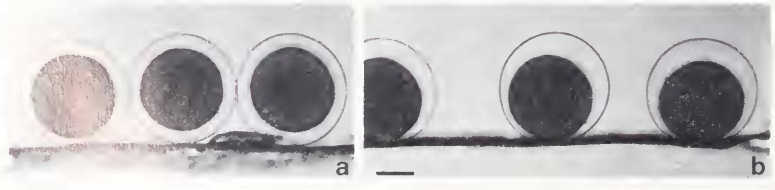

FIGURE 1. Normal fertilized eggs in horizontal view (a) and fertilized eggs centrifuged at 125 g for 3 min (b). *H. pulcherrimus*. Scale bar = 40 μm.

FIGURE 2. Photomicrograph of perivitelline fibers (arrow) observed under differential interference optics. Bar = 10 μm.

Effect of Ca-free sea water

Fertilized eggs were transferred into Ca-free artificial sea water one min after fertilization and washed with Ca-free sea water three times. After this treatment, no fibers formed in the perivitelline space (Fig. 3a). Correspondingly, the egg rested on the bottom of the perivitelline space in horizontal view.

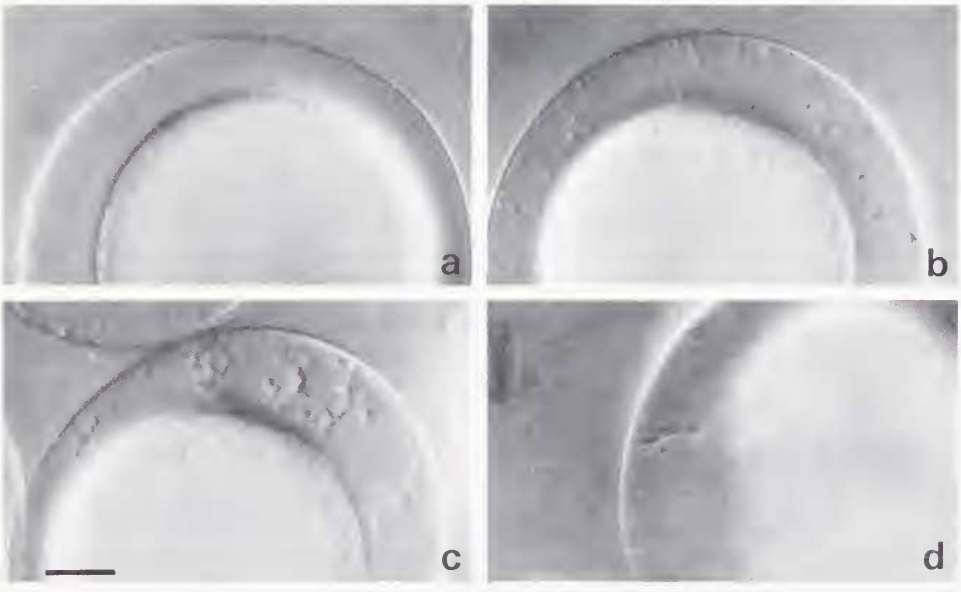

FIGURE 3. a: a fertilized egg transferred to Ca-free sea water one minute after fertilization. b: a fertilized egg transferred to normal sea water from Ca-free sea water (10 min after transfer). The fertilized eggs were immersed in Ca-free sea water for 30 min. c: 20 min after transfer. d: 60 min after transfer. A thick fibrous structure is observed. *H. pulcherrimus*. Bar = 20 μm.

When fertilized eggs kept in Ca-free sea water for 30 min were transferred into normal sea water, many granules soon appeared within the perivitelline space (Fig. 3b). Successively the granules were aggregated and arranged radially (Fig. 3c), which were eventually transformed into the thick fibrous structure (Fig. 3d). This fibrous structure begins to appear some 30 min after the transfer to normal sea water. By this time, half the eggs came to the center of the perivitelline space (Fig. 4). At 90 min after the transfer, most of the eggs were situated at the center of the perivitelline space. Such invariable coincidence of the perivitelline fibers and the concentric position of the egg shows unequivocally that the fibers are the supporting structure.

Electrophoretic movement of eggs within the perivitelline space

When fertilized eggs that are prevented from moving by slight compression with a cover glass are subjected to electrophoresis, the eggs migrate to the anodal side within the perivitelline space (Oshima, 1982). After the egg was shifted to the anode, the electric current was shut off. As shown in Figures 5 and 6, the egg slowly returned to its original position. This suggests that the fibers supporting the egg are not broken down but only stretched by electrophoretic movement of eggs. Observation by differential interference microscopy confirmed this hypothesis (Fig. 7). The fibers at the anodal side appeared to be bent and pressed by the egg against the wall of the fertilization membrane. If the fertilized eggs whose fibers had been broken by centrifugation (125 g, 3 min) were subjected to electrophoresis, eggs once shifted to the anode did not show any movement after the removal current. Therefore, the recovery to the concentric position is achieved by the fibers.

Analyses of electrophoretic movement of eggs within the perivitelline space

Electrophoretic movement of the egg in the perivitelline space is shown in Figure 8. The velocity of the egg near the center of the perivitelline space is fairly constant. In the experiments to follow, the speed determined near the center of the perivitelline space was taken as the velocity of the egg. After centrifugation of the eggs, the speed of the egg became greater than before centrifugation, as was expected (Table I): the lower speed in intact eggs will be due to the existence of the fibers within the perivitelline space. The constancy of speed as seen in Figure 8 suggests that the

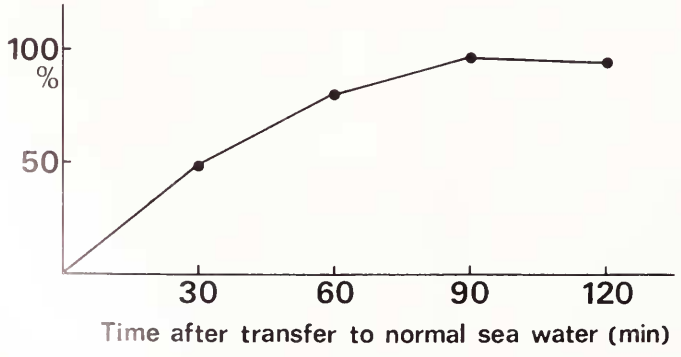

FIGURE 4. Gradual recovery in percentage of eggs situated at the center of the perivitelline space due to transfer to normal sea water after treatment with Ca-free sea water for 30 min. Fifty eggs were used to determine each point. Eggs were obtained from the same animal. *A. crassispina*. 26°C.

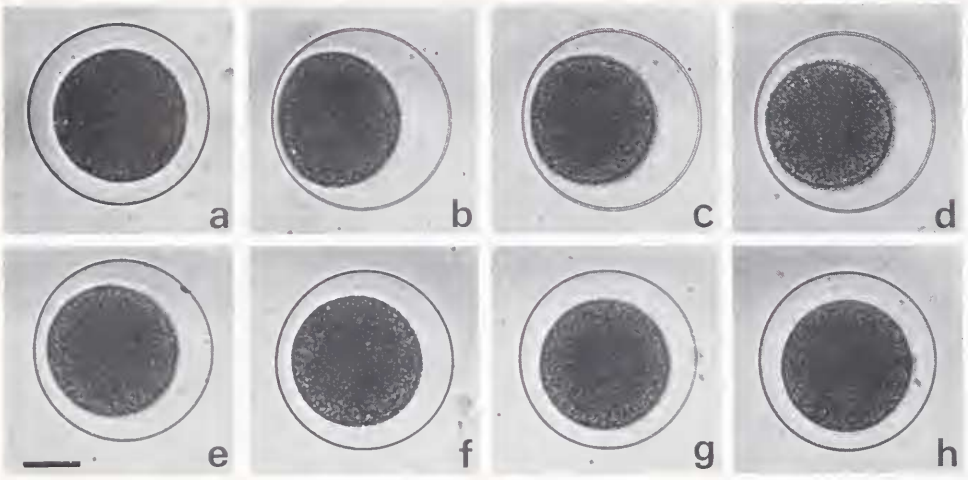

FIGURE 5. Return of egg to its original position after the removal of current. a: control. b: just before the removal of current. c: 25 s after the removal of current. d: 1 min. e: 2.5 min. f: 4 min. g: 7 min. h: 11 min. Potential gradient: 5.5 V/cm. *H. pulcherrimus.* Bar = 40 μm.

resistance to stretching by the perivitelline fibers is due mainly to its viscous properties rather than to its elastic properties, if any.

The relationship between the velocity of the egg and the potential gradient in *A. crassispina* is shown in Figure 9. The velocity of the egg in centrifuged eggs (without fibers) is proportional to the potential gradient. This fact indicates that the perivitelline fluid behaves as a simple Newtonian fluid, which obeys Stokes' law. The constant of the proportionality is about 3.8 (V/cm)/(μm/s).

In the presence of intact fibers in normal eggs, the results are somewhat complicated. The egg did not move with a potential gradient of 0.5 V/cm. Extrapolation

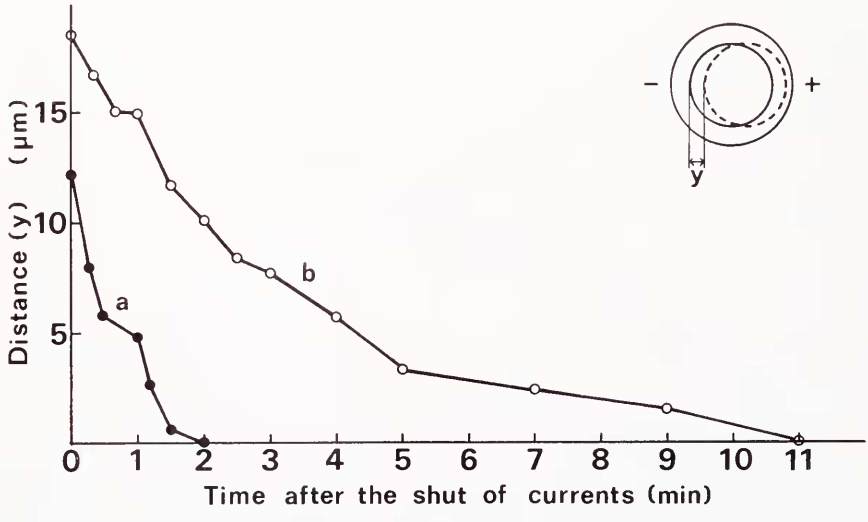

FIGURE 6. Return to the central position of the egg after cessation of electric current. a: *A. crassispina.* 2.7 V/cm for 15 s at 26°C. b: *H. pulcherrimus.* 5.5 V/cm for 15 s at 21°C.

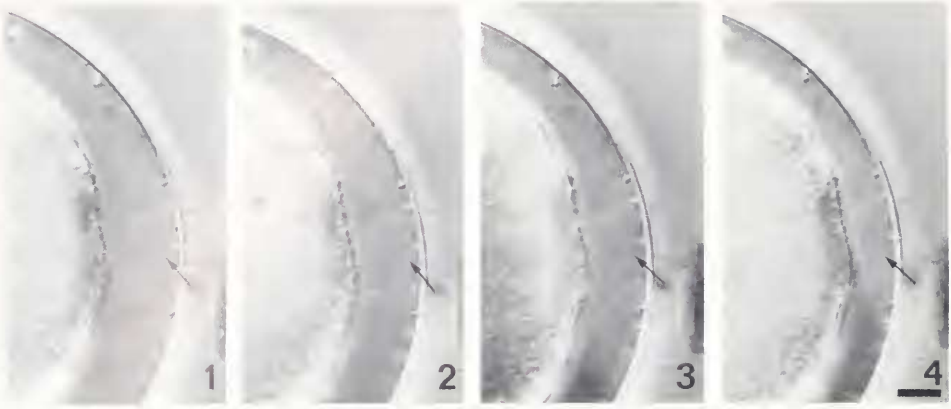

FIGURE 7. Recovery in length of perivitelline fibers (arrows) after the removal of current. *H. pulcherrimus*. Bar = 10 μm.

of the line (Fig. 9) indicates that a threshold for the movement of the egg lies at 0.65 V/cm. Such a 'threshold' indicates the presence of 'resting tension' of the fibers which is counterbalanced by the electrophoretic force of the potential gradient of 0.65 V/cm. This component of resistive force which is independent of stretch may be regarded as due to some surface force or tension at the protoplasmic surface of the fibers.

As seen from Figure 9, the slope of the line of centrifuged eggs (without fibers) is greater than that of normal eggs with intact fibers. This indicates that the fibers show a resistance proportional to the velocity of the eggs (or the speed of stretch of the fibers). This may be regarded as viscous resistance which the fibers show against their stretch. In conclusion, the resistive force due to the fibers is composed of tensional force and internal viscous resistance.

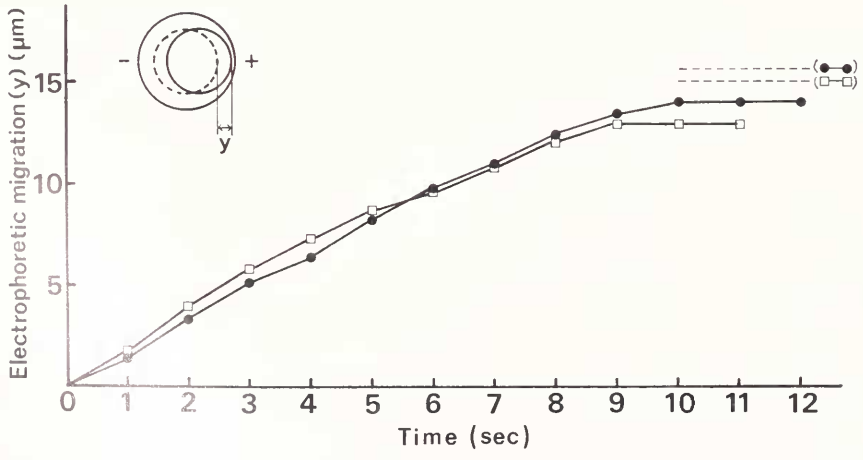

FIGURE 8. Electrophoretic migration of the egg with intact fibers. Two examples are shown. Broken lines are the boundaries of the perivitelline space. Potential gradient: 5.5 V/cm. *H. pulcherrimus*. 22°C.

TABLE I

Migration speed of the egg before and after centrifugation (125 g, 3 min)

Species	Velocity of egg		Temperature
	Before centrifugation	After centrifugation	
A. crassispina	1.06 ± 0.042	1.44 ± 0.067	26°C
H. pulcherrimus	1.47 ± 0.099	2.09 ± 0.143	22°C

Average and standard deviation based on 20 measurements are given in μm/s. Potential gradient: 5.5 V/cm.

Estimation of tension and internal viscous resistance of the fibers

To estimate tensional force and internal viscous resistance of the fibers, the magnitude of external force required for movement of the egg within the perivitelline space was determined by the following procedures.

After centrifugation (125 g, 3 min), the egg velocity falling within the perivitelline space under gravitational force was determined in sucrose-sea water solution following Hiramoto's method (1954). The time required to pass the distance of 5 μm (*A. crassispina*) or 10 μm (*H. pulcherrimus*) at the center of the space was measured 20–50 min after centrifugation and the velocity was determined. The average of ten measurements in *Anthocidaris* eggs and in *Hemicentrotus* eggs are 0.06 and 0.27 μm/s, respectively.

The gravitational force (F) acting on the egg is given as

$$F = \frac{4}{3}\pi a^3(D - d)g, \qquad (1)$$

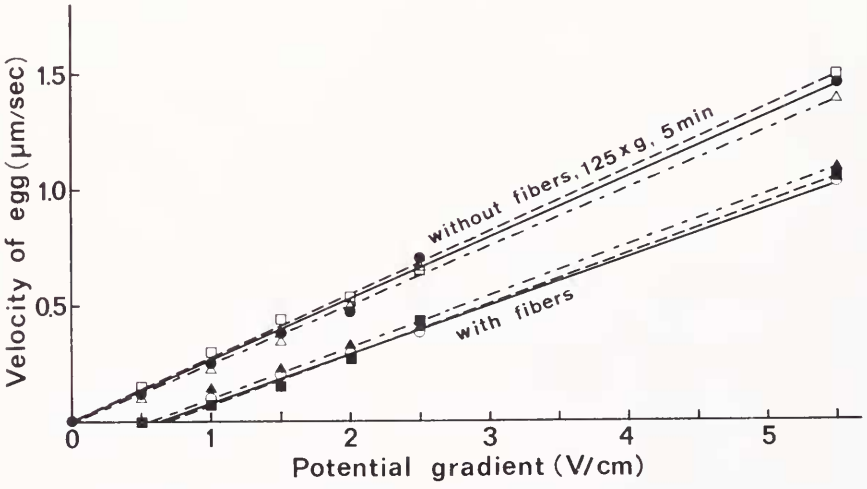

FIGURE 9. Relation between the velocity of egg and the potential gradient. Each point represents the average of five measurements. Results obtained from the same egg are shown by the same symbols. The results shown by the same sort of line were obtained from the eggs of the same animal. *A. crassispina*. 26°C.

where a is the radius of the egg; D is the density of the egg; d, the density of the perivitelline fluid; and g, the acceleration due to gravity. a was about 4.7×10^{-3} cm in *Anthocidaris* eggs, and 4.5×10^{-3} cm in *Hemicentrotus* eggs. The density of the egg (=D) without the fertilization membrane was determined by finding a sucrose-sea water solution isopycnic to the egg. The average value was 1.070 g/cm^3 (*A. crassispina*), and 1.066 g/cm^3 (*H. pulcherrimus*). Similarly, the density of intact fertilized eggs with the fertilization membrane was determined. From these densities and the volume of the egg, the density of the perivitelline fluid (=d) was estimated (*cf.* Hiramoto, 1954) to be 1.066 g/cm^3 (*A. crassispina*), and 1.059 g/cm^3 (*H. pulcherrimus*). Using equation (1), the values of 3.1×10^{-6} dyne (*A. crassispina*) and 5.6×10^{-6} dyne (*H. pulcherrimus*) were obtained for the gravitational force (F) acting on the egg without the fibers, which is counterbalanced by the resistive force due to the perivitelline fluid. From the above results, the external force required for the movement of the egg without the fibers at a rate of 1 μm/s is estimated to be 5.2×10^{-5} dyne in *A. crassispina,* and 2.1×10^{-5} dyne in *H. pulcherrimus.* From this value for *A. crassispina,* together with the proportionality of 3.8 (V/cm)/(μm/s) in Figure 9, the electrophoretic force under unit potential gradient (=1 V/cm) turns out to be 1.4×10^{-5} dyne ($=5.2 \times 10^{-5}/3.8$). The 'threshold' potential gradient, 0.65 V/cm, for the electrophoretic shift of the intact egg with the fibers corresponds to 0.9×10^{-5} dyne, being three times as high as the gravitational force acting on the eggs of *A. crassispina* ($=3 \times 10^{-6}$ dyne). In other words the fibers are stiff enough to support the egg.

Figure 9 was redrawn schematically in Figure 10. In normal fertilized eggs with the fibers, the results mentioned above are summarized by the following expression. External force = A (tension of the fibers) + B (internal viscous resistance of the fibers) + C (viscous resistance due to the perivitelline fluid).

In *Anthocidaris* eggs with the fibers, the above expression is written as external force = 0.9×10^{-5} (dyne) + 1×10^{-5} (dyne) $\times$ V + 5.2×10^{-5} (dyne) $\times$ V, where V is the migration speed of the egg in μm/s.

Discussion

In intact fertilized eggs with perivitelline fibers, the external electrophoretic force acting on the eggs was shown to be counterbalanced by the total of tensional force, internal viscous resistance of the fibers, and viscous resistance due to the perivitelline fluid. After removal of the current, the eggs that were shifted to anodal side returned to their original positions. Perhaps this is due to tensional force of the fibers at the cathodal side. In this case, tension of the fibers counterbalanced the sum of internal viscous resistance of the fibers against shortening and viscous resistance due to the perivitelline fluid, or tension of the fibers = 1×10^{-5} (dyne) $\times$ V + 5.2×10^{-5} (dyne) $\times$ V (*A. crassispina,* see above section). As seen in Figure 6, the initial return velocity (taken as the mean velocity during first one min in this study) is about 0.12 μm/s in *Anthocidaris* eggs. Substituting 0.12 (μm/s) to V in the above equation we have 0.7×10^{-5} dyne for the tensional force, which agrees with 0.9×10^{-5} dyne estimated in Results on the basis of the speed of electrophoretic migration of the egg.

To determine the total number of the fibers existing within the perivitelline space, five sheets of microscopical photographs were taken successively at uniform intervals of 2.6 μm near the largest optical section of eggs. From the series of the photographs, the number of fibers existing in the perivitelline space of about 10 μm thick (surface area around this space corresponds to about 10% of the total

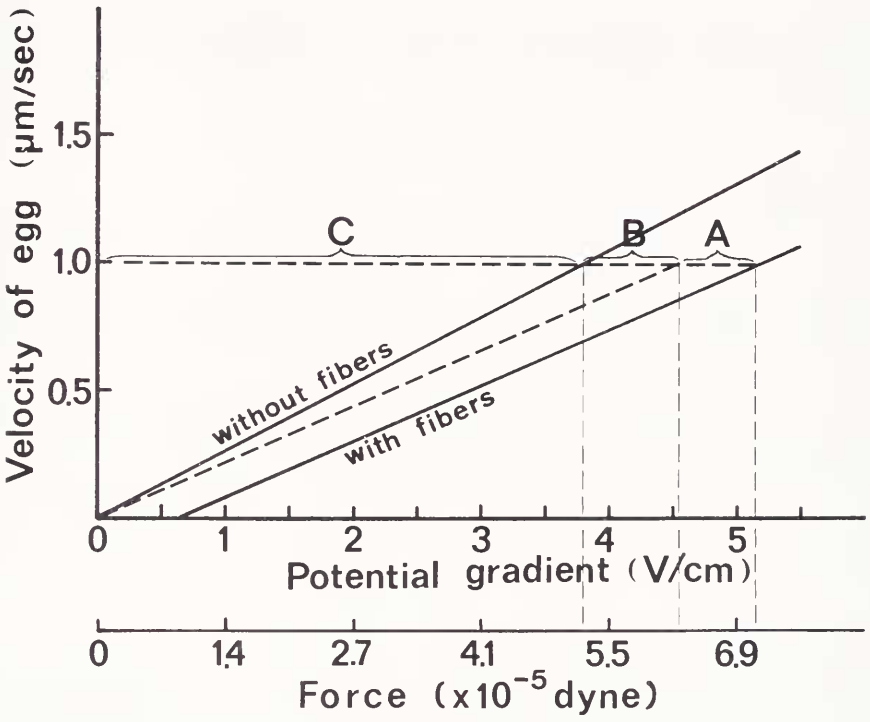

FIGURE 10. Generalized schematic reproduction of Figure 9. A: resistance due to tension of the fibers. B: internal viscous resistance of the fibers. C: viscous resistance due to perivitelline fluid.

surface area of eggs) in which a largest optical section of eggs is located was estimated. In three eggs, the numbers were 108, 148 and 180. Therefore, 1000–1800 fibers will exist in the whole perivitelline space of an *Anthocidaris* egg.

When the electrophoretic force acting on the eggs was below 0.9×10^{-5} dyne, the eggs did not show any movement. If the fibers within the stereoangle of 60 degrees at the cathodal side are effective to support the egg, the number of these fibers is estimated to be 6.7% ($=(2 - \sqrt{3})/4$) of the total fibers. That is, about 100 fibers will practically support the egg, if the number of the total fibers is 1500. This indicates that a single fiber could withstand the stretching force of 1×10^{-7} dyne. The nature of the structure responsible for such a resistance to stretching is still not clear, but if the resistance is due to the tension working at the protoplasmic surface of the fiber with the diameter perhaps as small as 0.1 μm, then the calculated tension will amount to 2.9×10^{-3} dyne/cm, which approximates to the value of surface tension of a protoplasmic droplet in *Nitella flexilis* (2.3×10^{-3} dyne/cm, *cf.* Kamiya and Kuroda, 1958).

The centrifugal force acting on the egg when it is centrifuged at 125 g was calculated. By using Hiramoto's data (1954) on the density of the egg in *H. pulcherrimus* (1.0715) and that of the perivitelline fluid (1.0369) determined in normal sea water, it came out to be 1.6×10^{-3} dyne. This is forty times greater than the electrophoretic force under the potential gradient of 5.5 V/cm that is the maximum gradient used in this study. By centrifugation at 125 g the fibers will be stretched much more rapidly than by electrophoresis, although their final length is the same as that in electrophoresis, due to a limit set by the presence of the fertilization

membrane. Thus, the breakdown of the fibers by centrifugation at 125 g for 3 min will be due to either (1) the rapid stretch or (2) the lapse of time in fully stretched state.

[Appendix]

Viscosity of perivitelline fluid

The resistive force (F) acting on a sphere moving in a fluid is given by Stokes' law, $F = 6\pi a\mu \cdot U$, where a and U are the radius and speed of the sphere, and μ is the viscosity of the fluid.

When the sphere is moving within a definite boundary, the force (F) is written as

$$F = 6\pi a\mu \cdot U \cdot K, \tag{2}$$

where K is a wall correction factor. For the sea urchin egg (with radius A) moving within a spherical perivitelline space with radius B,

$$K = \frac{1 - \lambda^5}{1 - \frac{9}{4}\lambda + \frac{5}{2}\lambda^3 - \frac{9}{4}\lambda^5 + \lambda^6}$$

(3, *cf.* Happel and Brenner, 1965), where $\lambda = A/B$.

The value of $\lambda(=A/B)$ is 0.84 in *Anthocidaris* eggs, and 0.78 in *Hemicentrotus* eggs. Calculation by equations (2) and (3) revealed that the viscosity of the perivitelline fluid in *Anthocidaris* and *Hemicentrotus* eggs are 0.021 and 0.024 poise ($1\ P = 10^{-1}\ Pa \cdot s$), respectively. These values, which are about twice as large as that of sea water, suggest the existence of some high-molecular substance in the perivitelline space.

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